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UNIVERSITY OF ALBERTA

COMPARISON OF ALTERNATIVE SWEETENING SYSTEMS IN FORMULATION OF COMMERCIAL WHEY BEVERAGE

by

Ceike H. Beukema

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE
IN
FOOD PROCESSING

DEPARTMENT OF FOOD SCIENCE

EDMONTON, ALBERTA
Spring, 1990

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The undersigned certify that they have read, and recommended to the faculty of Graduate Studies and Research for acceptance, a thesis entitled **Comparison of alternative sweetening systems in formulation of commercial whey beverage** submitted by **Ceike H. Beukema** in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in FOOD PROCESSING

Dr. P. Jelen (Supervisor)

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Dr. R. Locock

Date March 22, 1990

To my Parents, Menne and Diedy Beukema.

ABSTRACT

The production of whey beverages is one of the more economically feasible options of whey utilization, however, a disadvantage of using whey as a base for the manufacture of a beverage may be the calorie content of whey, largely attributable, to its high lactose content (5%). Aspartame (apm) and acesulfame-K are high potency sweeteners that don't exhibit characteristics such as aftertaste or bad mouthfeel, therefore, they may be suitable as total sugar replacements in whey beverages, resulting in a significant reduction of the amount of carbohydrate present. Lactose hydrolysis could also be used to minimize the amount of sweetener required.

In this work, the sensory and heat stability aspects of the two high potency sweeteners, apm and acesulfame-K were evaluated for use in a whey-based beverage. It was determined that the amount of each sweetener required to produce a sweetness level equal to that of a beverage containing 10.5 percent invert sugar was 0.25 and 0.275 grams per litre of apm and acesulfame-K, respectively. When used together, apm and acesulfame-K appeared to exhibit synergism, resulting in a twenty-five percent reduction in the amount of sweetener required to maintain the equisweetness level of the product.

The enzymatic hydrolysis of 90% of the lactose present in whey was found to increase the sweetness of the apm- or

acesulfame-K- sweetened product, thereby reducing the amount of high potency sweetener required by twenty-five to fifty percent.

A complementary study was carried out to determine if either of the two high potency sweeteners was affected by heat during the processing of the low-pH beverage. The results showed that, following a heat treatment of 30 minutes at 90°C, no decrease in the sweetness or other taste qualities were attributable to either apm or acesulfame-K and, analytically, only a slight degradation, 13.2%, of the apm was noted. Acesulfame-K was found to be totally unaffected by the heat treatments to which it was subjected.

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1. INTRODUCTION

Whey, a valuable by-product of the cheese-producing industry, is still considered a serious problem with regards to utilization. To most Canadian dairies, cottage-cheese whey has no further use after cheese production and is discarded, resulting in high sewage costs. These costs are a function of quantity, as up to ninety percent of the milk used for the production of cheese remains as whey and is eliminated (Jelen, 1979) and of the Biological Oxygen Demand(BOD)-value of whey, which is very high (Pico, 1976). In New Zealand and many other countries, excess cheese whey is used as animal feed and soil fertilizer (Mann, 1987).

A great deal of effort has been exerted utilizing cheese whey as an ingredient in palatable and nutritious products for human consumption. Processes of concentrating and modifying cheese whey have been implemented in economical and useful techniques such as spray drying and ultrafiltration (Coton, 1985). However, these processes are economically feasible only for industries which produce very large amounts of whey, since these processes require large investments towards equipment. In the Netherlands, where the production of cheese is a major part of the dairy industry, special whey processing plants have

been designed to extract and process the valuable components of this product. The resultant products are most often used in baby formulas and as nutritional enhancers in many other food products (Short, 1978). In addition, whey is used for several non-food purposes such as pharmaceuticals, resins, solvents, coatings, and acrylic plastic (Kosaric and Asher, 1982). Spray drying and ultrafiltration of whey is not an option for smaller dairies and therefore other means of utilization must be sought. The production of Mysost, a Norwegian type of whey cheese (Jelen and LeMaguer, 1986) and whey drinks (Holsinger *et al.*, 1974) may be economically feasible for smaller dairies, because the manufacturing of these products, in most cases, does not require special equipment. A review by Kravchenko (1987) shows that vast amount of research has been carried out on development of whey beverages in the past. The advantages of using whey as a beverage include: (1) whey can be used directly from the cheese vat eliminating disposal problems; (2) the process of manufacturing whey drinks is simple and may not require special equipment. A possible disadvantage of using whey as a base for the manufacture of beverages is the high calorie content of whey, attributable to the high amount of lactose (5%). In order to obtain a palatable product with desirable sweetness contributed by nutritive sweeteners the

total carbohydrate content of the whey drink is high, in comparison with fruit juices and soft drinks (Table 1.1).

Table 1.1. Caloric values of various beverages*

beverage	caloric value (kcal/100mL)
whey beverage	58
sprite	48
apple juice	47
grape juice	61

*Adapted from USDA, 1982; Jelen *et al.*, 1987.

Previous experiments with artificial sweeteners in whey beverages resulted in products having a bitter aftertaste therefore being less acceptable than original formulations (Dwivedi, 1978). Since aspartame (apm) and acesulfame-K (SUNETTE®) are high potency sweeteners without exhibiting problems such as aftertaste and bad mouthfeel (Larson-Powers and Pangborn, 1978; Lueck, 1981), they may serve as a total sugar replacement in whey drinks reducing the amount of

carbohydrate present remarkably.

According to Holsinger *et al.* (1974), whey drinks can be classified into four groups: (1) beverages from whole whey; (2) non-alcoholic beverages from deproteinized whey; (3) alcoholic beverages from whey; (4) protein beverages. The processes involved in the manufacture of each type are quite different.

No additional treatment, except addition of flavor and sweetener followed by pasteurization, may be necessary for beverages in group 1. Group 2 involves the heat treatment of whey to 90°C and filtration or centrifugation in order to remove the coagulated whey proteins, with the clear supernatant after centrifugation being used as a base for the beverage. Alternatively, ultrafiltration can be used for the separation of whey proteins. For group 3, the whey is usually deproteinized and fermented. The final products in this group include wine, beer and several types of "cooler". Most beverages in group 4 have an approximate pH of 7 and may contain up to 50% milk. However, recent innovations in yogurt drinks with added whey have resulted in a line of new products with a pH of 4.2 - 4.5.

In many Western European nations these whey-based beverages, in particular the fruit flavored varieties, have gained a great deal of popularity owing to their refreshing taste and

healthful image. With the use of artificial sweeteners such as aspartame and acesulfame-K, the low calorie- and calorie-reduced potential of a whey-based product is enormous.

1.1. RESEARCH OBJECTIVES

The main research objective was to investigate the sensory effects of using the artificial sweeteners acesulfame-K and aspartame (apm) in a whey-based fruit beverage. Specific objectives included: determining the amount of the two sweeteners required to replace the commonly used sugar; determining the synergistic effect of acesulfame-K and apm on sweetness in the beverage when used jointly; determining if hydrolysis of the lactose present in whey results in a further possibility to reduce the amount of added sweetener; and determining if acesulfame-K and apm are affected by heat in the processing of the low-pH whey beverage.

2. REVIEW OF LITERATURE

2.1. WHEY

2.1.1. Composition of whey

During the manufacture of cheese, the coagulated fraction of milk, which contains casein and most of the milk fat, is recovered.

The remaining liquid portion is known as whey. Sweet whey results from the manufacture of products such as cheddar and mozzarella that principally use rennet type enzymes at a pH of approximately 5.7 - 6.2. Acid whey is the by-product of manufacture of cottage cheese, quark, and other fresh cheeses where the coagulum is formed by acidification in a pH range of 4.5 - 4.8. In this work, cottage cheese whey was used. Table 2.1 lists the proximate composition of acid and sweet wheys.

2.1.2. Whey processing

A large proportion of liquid whey has been used, traditionally, as animal feed or discarded as effluent. More

Table 2.1. The average composition of whey

Constituent	sweet whey (Cheddar cheese)	acid whey (Cottage cheese)
	(% w/w)	(% w/w)
water	93.7	93.5
lactose	4.95	4.90
lactic acid	0.05	0.40
fat	0.5	0.04
protein	0.8	0.75
ash	0.5	0.8
pH	5.7 - 6.3	4.5 - 4.6

Adapted from Coton (1976).

recently, however, whey and whey-based products have found uses as ingredients in the food industry. Uses for whey include dried whey powder and dried whey protein concentrates as added protein in baby foods, ice cream, baked goods, cooked foods (such as gravies), and confections (Palmer, 1980). Hydrolyzed lactose from whey can be used as a sweetening agent or with whey

proteins to permit faster browning of baked goods.

Small quantities of whey have been used as the base for whey drinks. Unit processes for the manufacture of products which involve whole whey or fractions of whey include: evaporation, reverse osmosis, ultrafiltration, ion exchange, fermentation and chemical modification. Table 2.2 gives an overview of whey utilization alternatives.

Table 2.2. Uses of whey

Form of whey	Market
liquid	animal/human
concentrated (evap/RO)	animal/human
dried (demineralized)	animal/human
hydrolyzed	human
anaerobic fermentation of whey	industrial
fractionated (UF/ion exchange):	
protein rich	human/industrial
lactose rich	human/industrial

Adapted from Coton (1985).

2.1.3. Lactose

The largest component of liquid whey (excluding water) is lactose, as shown in table 2.1. The amount of lactose found in cow's milk is approximately 4.8%, however, in other species of mammal, it can vary from less than 2.0 to 7.0 percent.

Lactose (4-ß-D-galactopyranosyl-D-glucopyranose) is a disaccharide consisting of glucose and galactose. Lactose is less sweet than its constituents, having a relative sweetness (sucrose = 1.0) of 0.4 compared to 0.6 for galactose and 0.7 for glucose (Tamime and Robinson, 1985). Lactose is much less sweet than fructose. For example, a 0.8% fructose solution has the same sweetness as a solution containing 3.5% lactose. Besides being less sweet, lactose is also less soluble than its constituent monosaccharides. The various properties of lactose have been reviewed in detail by Nickerson (1974). As cottage cheese whey contains 4.9% lactose, (Table 2.1) it is understandable that in a beverage containing 90% whey, lactose would be a significant component. However, lactose is not always a desired substance in food products, particularly for the consumers who are lactose intolerant. The human body cannot absorb lactose directly from the intestine, and therefore the lactose must be broken down into its constituent

monosaccharides, glucose and galactose, which can be absorbed (Nijpels, 1982). Some people possess the ability to secrete the enzyme lactase so that lactose can be readily broken down. However, there is a small percentage of Caucasian people and most Africans and Asians that lack the ability to secrete lactase and as a result suffer from cramps and diarrhea upon the ingestion of lactose containing foods (Kelley, 1984). For this reason, and to utilize the greater sweetness of the constituent monosaccharides of lactose, glucose and galactose, the process of lactose hydrolysis may be implemented.

2.1.4 Application of lactose hydrolysis

Lactase, also known as ß-D-galactoside galactohydrolase (E.C.3.2.1.23) is an enzyme which acts specifically on lactose. It breaks the ß-1-4 glycosidic linkage and liberates the monosaccharides glucose and galactose. Lactase can be found in plants, fungi, bacteria, yeasts and the intestine of young mammals (Nijpels, 1982). Details of the theory as well as the practical applications of lactose hydrolysis have been reviewed by Shukla (1975) and Ryder (1987). Applications include such processes as the use of free, immobilized or reactor enzymes, direct or ion exchange-catalyzed acid hydrolysis and, finally, the

use of hydrogen gas in the presence of a nickel catalyst. The economic implications of each industrial application must be considered by the individual producer. In small scale operations where only low amounts of lactose-hydrolyzed whey are required, the use of "throwaway" enzymes may be the most economically feasible option. However, as the scale of operation rises, it is more likely that an immobilized system would be optimal (Ryder, 1987).

2.1.5. Whey-based fruit beverages

It appears that there is growing importance of whey in the beverage market as much research is being devoted to the development of whey-based fruit drinks (Mann, 1988). Prendergast (1985) reported that whey-fruit beverages have lighter mouthfeel, are less astringent, and are more nutritional than conventional fruit drinks. The main beneficial components of whey are L(+) lactic acid, phosphates, calcium, vitamins B1, B2, B6, and amino acids. Citrus flavors and citric acid tend to mask much of the whey flavor and are therefore the most suitable materials for flavoring and pH-adjustment of whey-based fruit drinks (Demott, 1974). Current examples of commercial whey beverages with fruit juice components include

'Kwink', 'Djoez', 'Yor', and 'Taksi' in The Netherlands. Jelen *et al.* (1987) provided information on compositional analysis of some of the commercial whey-based fruit beverages.

2.2. SWEETNESS AND SWEETENERS

Sweeteners have been used to increase the palatability of foodstuff since prehistoric times, following the discovery of honey in 2600 B.C. (Newsome, 1986). The earliest reference to sugar and sugar cane is recorded in a scroll from the year 375 A.D. By the 14th century, the Arabs had developed the first sugar cane refining processes (Newsome, 1986). Research has shown that people have an inborn desire for sweet taste; a response which has been shown to be an innate reflex rather than a reaction acquired by conditioning or learning (Watson, 1971). Today, sweeteners of one kind or another are found in the diets of most, if not all, individuals. An adult human consumes approximately 40 Kg of sugar per year (Heijden, 1988). This may be added to the diet in the form of table sugar (sucrose) but is more often added during food processing at the industrial level. Sweeteners used in the manufacture of processed foods exist in many various forms, including fructose (levulose), glucose (dextrose), glucose/galactose syrup, invert sugar, sorbitol,

honey, saccharin, cyclamate, aspartame, and acesulfame-K (Tamime and Robinson, 1985). Each of the sweeteners available has specific applications and limitations depending on which type of food it is used in, processing conditions of the food, and other factors. Any sweetener which provides energy is considered a nutritive sweetener, therefore, sugars, sugar alcohols, sugar syrups and honey are all classified as nutritive sweeteners. Aspartame (apm) is also considered a nutritive sweetener since it provides the same amount of calories per gram as sugar. However, apm is approximately 200 times sweeter than sucrose, therefore it contributes an insignificant amount of calories to the foods it sweetens. Acesulfame-K on the other hand, is classified as a non-nutritive sweetener, because it is not metabolized by the body and does not contribute any energy to the diet.

Saccharin, an organic petroleum compound discovered in 1879, and cyclamate, discovered in 1937, are also classified as non-nutritive sweeteners (Newsome, 1986). Table 2.3 lists the relative sweetness of these high potency sweeteners in commercially prepared soft drinks as well as their acceptable daily intake (ADI) levels. This is the amount, in grams per kilogram body weight, one can use without any health risk.

When comparing the sensory properties of sweeteners, it is

important to distinguish between the two different aspects; taste quality and sweetness intensity. Taste quality is the ability of a high potency sweetener to exhibit as clean and refreshing a taste as that of sugar without any bitter flavor or lingering aftertaste. The intensity of a high potency sweetener is very important because the sweetening effect of these sweeteners is extreme and very easily perceived. Temperature, concentration, and nature of the food product being sweetened are some of the factors influencing the sweetness intensity of these high potency sweeteners.

Table 2.3. Comparison of sweetness and ADI levels (WHO/FAO) of four intensive sweeteners

sweetener	relative sweetness (sucrose = 1)	ADI (mg/kg body wt.)
saccharine	300	2.5
cyclamate	30	11.0
acesulfame-K	200	9.0
aspartame	200	40.0

Adapted from Heijden, (1988).

2.3. ASPARTAME

Aspartame (apm) is a high potency sweetener known by the trade name Nutrasweet®. The compound is the 1-methyl ester of the dipeptide N-L-aspartyl-L-phenylalanine. The sweet taste of N-L-aspartyl-L-phenylalanine-1-methylester was discovered in 1965 by James Schlatter, a G.D. Searle and Co. chemist (Mazur, 1984). Following the discovery of this new compound, Searle's laboratory examined approximately 200 analogs of the substance but commercialized the original compound because it had the highest sweetness potency and taste quality and could also be manufactured the most economically (Mazur, 1984). This new sweetener has emerged under the name aspartame and is covered by many patents.

2.3.1. Synthesis of Aspartame

The synthesis of aspartame begins with the free amino acids phenylalanine and aspartic acid. Phenylalanine is methylated and coupled with aspartic acid through a series of chemical reactions resulting in a final product which is crystallized and dried (Beck, 1978).

2.3.2. Properties

Aspartame is a white, odorless, crystalline powder that has a clean, sweet taste. apm is a sweetener as well as a flavor enhancer to be used in foods and beverages (Baldwin and Korschen, 1979). Since apm is a dipeptide, its caloric value is the same as protein; 4 kcal/g. Unlike bulk carbohydrate sweeteners such as sucrose, apm provides only sweetness and cannot impart other physical properties such as body or water binding ability. Because the concentration of sweetness of apm is so high (Table 2.2) apm can be used in quantities such that it won't dilute the essential nutrients of some foods and can reduce the amount of calories up to 95%. Studies on sensory evaluation have shown that apm appears to enhance the flavor of some foods; this enhancement being the most significant with naturally derived flavors rather than those artificially manufactured (Baldwin and Korschen, 1979; Homler, 1988). When apm is combined with other high potency or carbohydrate sweeteners, it's usefulness becomes apparent. Carbohydrate sweeteners such as fructose, sucrose or glucose maintain their sugar-like taste with a desired decrease in calories when apm is used in combination with them. When apm is combined with high

potency sweeteners, such as cyclamate, saccharin or acesulfame-K that have a bitter aftertaste, the bitterness is masked with a degree of improvement reflecting the higher proportion of apm used with the sweeteners. This synergy is dependent upon the proportion of each sweetener and type of food system (Bakal, 1983; Moskowitz *et al.*, 1978; Porter, 1983).

2.3.3. Stability

The stability of dry apm is evident as temperatures well over 150°C (305°F) are needed for its breakdown to become substantial (Homler, 1988). These conditions are not likely to occur during the manufacture or storage of dried food products, however, under moist manufacturing and storage conditions apm is unstable and its decomposition appears to follow first-order kinetics (Beck, 1978). In aqueous solutions such as soft drinks with pH 3.4 or less and at 40°C or higher, the ester bond of apm is hydrolyzed forming the dipeptide aspartylphenylalanine and methanol. Alternatively, methanol may be generated by the cyclization of apm leading to the formation of diketopiperazine (DKP) (Figure 2.1).

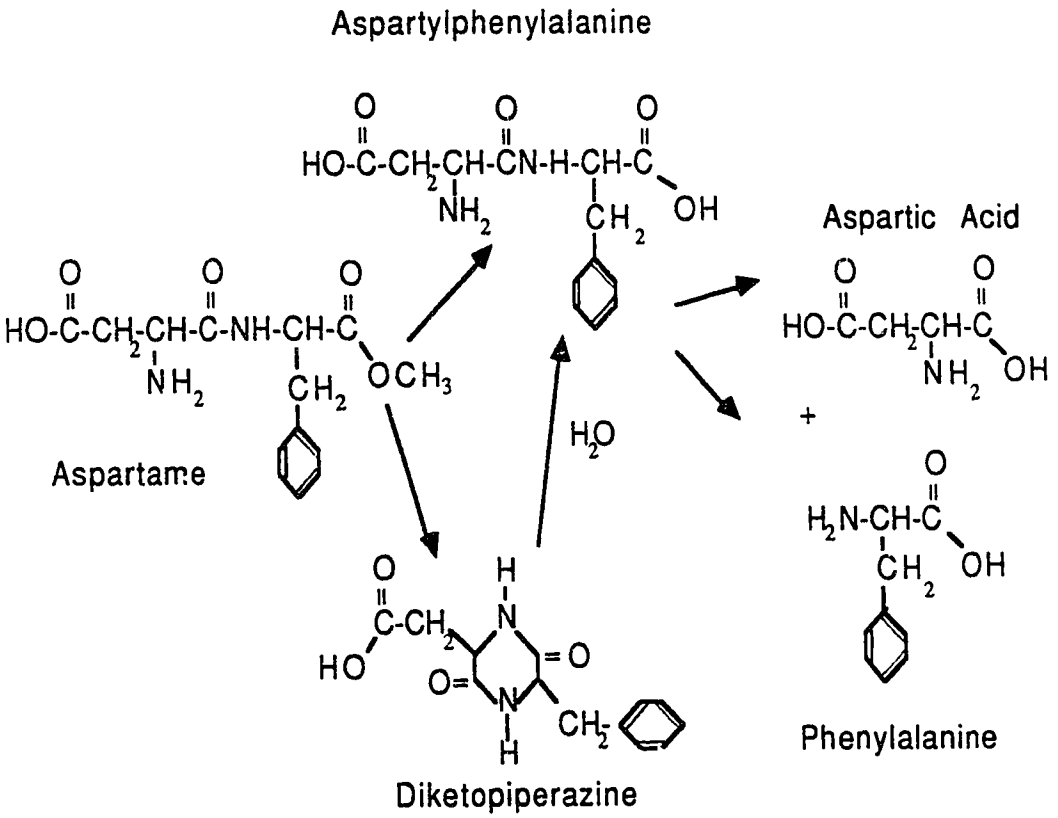

Figure 2.1. Aspartame and its principal conversion products (adapted from Homler, 1988).

Further hydrolyzation of aspartylphenylalanine leads to the production of its component amino acids aspartate and phenylalanine. Diketopiperazine, aspartylphenylalanine, aspartate and phenylalanine are not sweet and in food, the formation of these compounds causes loss of perceived sweetness; however, no off-flavor or color is noticed (Homler, 1988). The combined effect of pH, temperature and time on the

stability of apm in water is shown in figure 2.2.

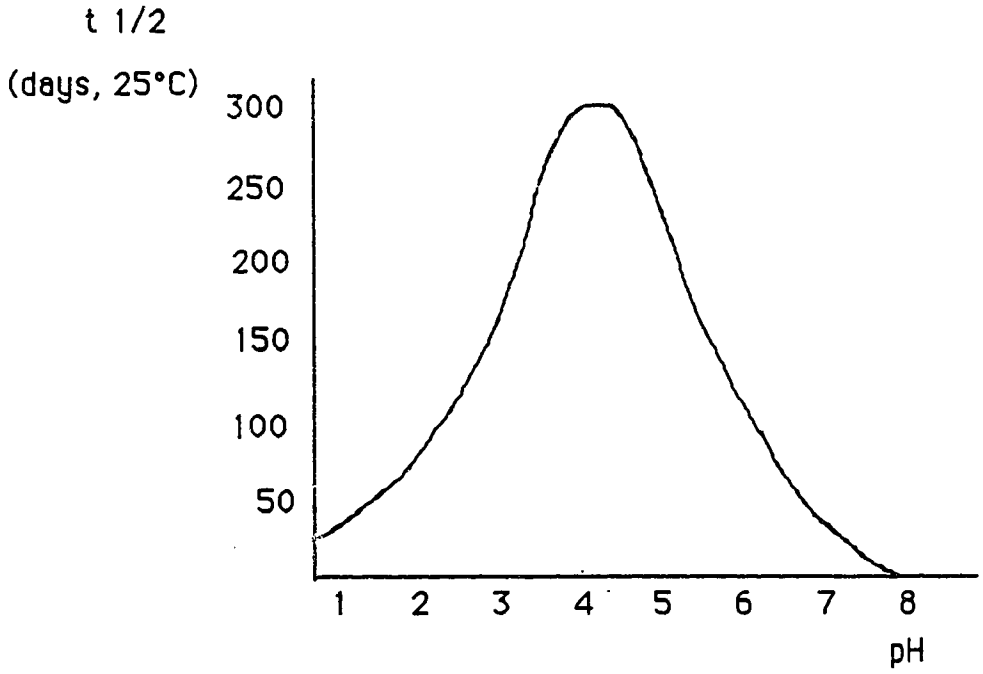

Figure 2.2. Half-life of apm in aqueous buffers at 25°C. (adapted from Mazur and Ripper, 1979).

APM is most stable between pH 3.5 and 5; at this pH level it can withstand High Temperature Short Time (HTST) or Ultra High Temperature (UHT) processing with minimal breakdown (Homler *et al.*, 1987; Andres, 1987), as shown in Figure 2.3.

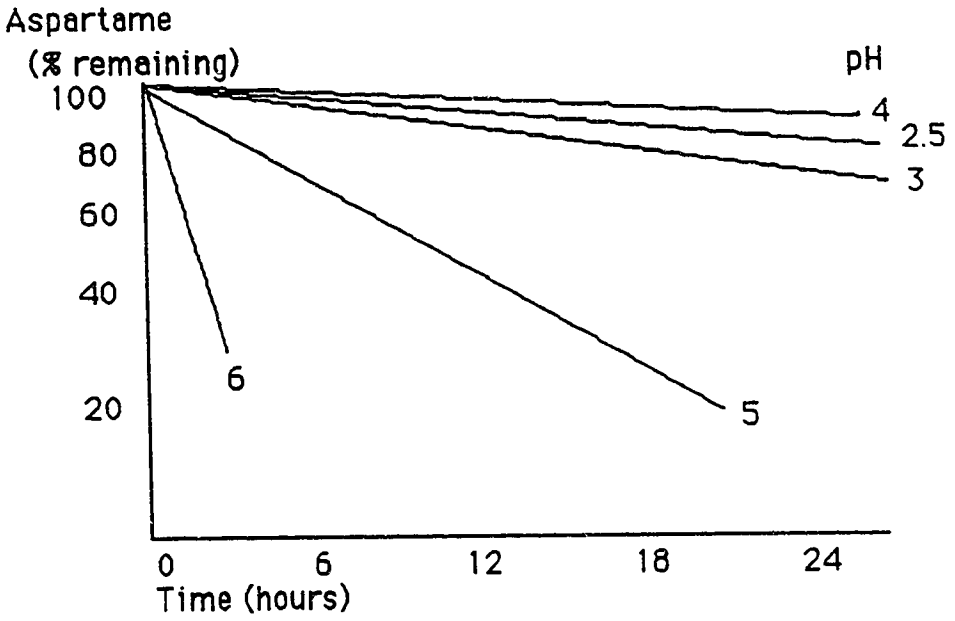

Figure 2.3. Stability of apm in aqueous buffer at 80°C. (adapted from Beck, 1978).

2.3.4. Sweetness

Because apm is 160 - 220 times sweeter than sucrose, only very small amounts are required for sweetening, depending on the flavor system, pH and tasting temperature (Beck, 1974). Unlike other artificial sweeteners, apm has a sweet taste of sugar without a bitter, chemical, or metallic aftertaste. APM has the same sweetness quality as fructose, glucose and D-sorbitol (Anon., 1989a).

2.3.5. Solubility

The solubility of apm is an important parameter, since it increases the applicability of this sweetener in such foods as beverage concentrates and dessert mixes. APM is slightly soluble in water (about 1% at 25°C) and sparingly soluble in alcohol (Beck, 1978). Aspartame is not soluble in fat.

The solubility of apm is a function of temperature and pH (Figure 2.4), increasing with an increase in temperature, and decreasing with an increase in pH above pH 3.0 (Beck, 1978).

2.3.6. Applications

APM is now permissible in a wide variety of products as numerous marketing, technological, and consumer concerns have been alleviated following many intense studies of this substance. Canada was one of the forerunning countries to approve the use of apm in diet foods (Newsome, 1986).

The chronology of approval of apm as sweetener in food products in various countries is listed in Table 2.4; the products in which this sweetener was first used are also shown.

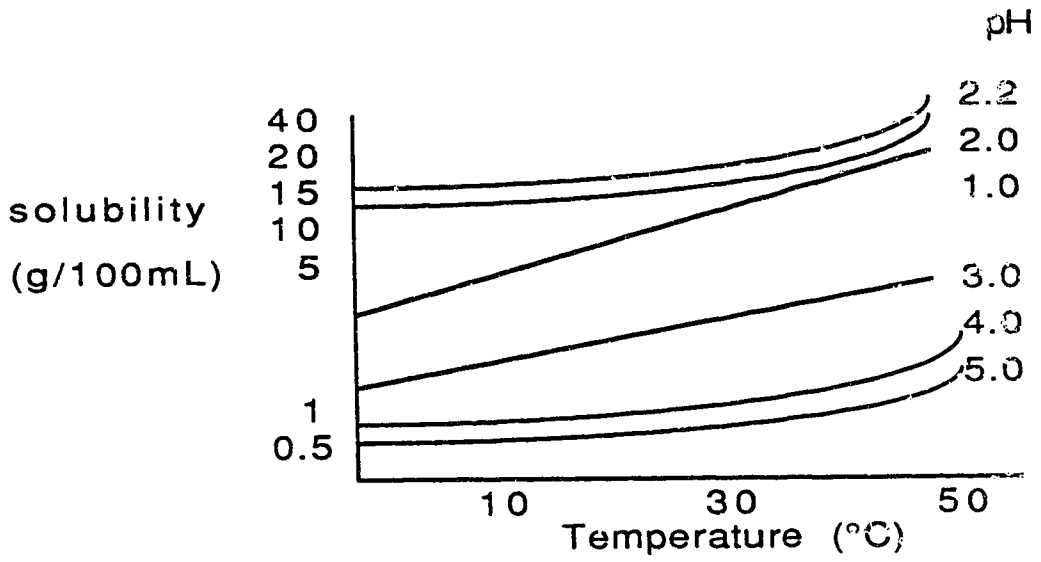

Figure 2.4. Solubility of apm in aqueous solutions at different pH and temperatures (adapted from Beck, 1978).

Table 2.4. The chronology of apm approval in some food products (Heijden, (1988)

Date	Country	Product
1981	Canada	table top sweeteners,

Table 2.4. The chronology of apm approval in some food products (cont'd.)

Date	Country	Product
		soft drinks
	USA	soft drinks
1982	Sweden, Switzerland, and Germany	table top sweeteners
1983	Holland, New Zealand, and Japan	table top sweeteners
	Denmark	soft drinks
	England	general use
1984	Sweden	cough drops, chewing gum
	Malta	soft drinks
	Belgium	table top sweeteners
1985	Sweden	ice cream, soft drinks
	Germany	diet yogurt
	Switzerland	diet products
1986	Germany	fruit preserves, puddings
1987	Holland	soft drinks, dairy products

Table 2.4. The chronology of apm approval in some food products (cont'd.)

Date	Country	Product
	Sweden	fruit yogurt, jam
	Spain	diet products
1988	France	dairy products, candy
	Belgium	yogurt, candy

2.3.7. Regulatory status

There are only a few other food additives that have received the kind of regulatory agency review as apm prior to its use in foods and beverages. The apm regulations have been the subject of public comment since mid 1974. The results of all of the reviews of apm have determined that this sweetener is a safe food additive for the general population. The World Health Organization (WHO) and the Food and Agriculturural Organization

(FAO) allocated an ADI (Acceptable Daily Intake) of 40.0 mg/kg body weight. For the benefit of persons with phenylketonuria (PKU) the presence of phenylalanine, a hydrolytic product of apm, is noted on the ingredient label of products containing apm (Janssen and Heijden, 1988). Depending on regulations of a specific country, apm is regulated as a 'sweetener', 'artificial sweetener', 'food additive' or by a combination of regulatory classes (Heijden, 1988). Most countries require that the name aspartame be shown and the amount of apm per serving (or other specified unit) be declared on the primary display label of the consumer product.

2.3.8. Safety

APM is one of the most extensively tested food additives in the history of food processing and technology. In terms of types and variety of experiments and in the dosage given to human volunteers, apm has been the subject of hundreds of intensive toxicology studies (Horwitz and Bauer-Nehrling, 1983; McCormic, 1974; Stegink and Filer, 1984).

When assessing the safety of a food additive, it is necessary to take into account the probable consumption level and compare that level with consumption levels potentially injurious to

human health. This has been done extensively with apm and its principal metabolites aspartate, phenylalanine and methanol. Studies have demonstrated that apm has a substantial margin of safety as do its metabolites, beyond any reasonable consumption level expected to be achieved by a normal human being (Stegink, 1987). Aspartate (aspartic acid) can be interconverted into the amino acid glutamate in the body. As very high levels of either one of these substances can damage the central nervous system, research was conducted to determine the effect of apm on the blood levels of these amino acids. Results of studies on adults and children have shown that with the consumption of normal and above normal (200 mg apm/kg body wt.) levels of apm, the plasma phenylalanine and aspartic acid concentrations will not rise to levels commonly associated with adverse effects (Filer *et al*, 1983; Frey, 1976; Janssen and Heijden, 1988). However, in individuals with the inherited disease phenylketonuria (PKU), phenylalanine is not metabolized properly and instead builds up in high concentrations in the blood and brain. These individuals are lacking the enzyme phenylalanine hydroxylase, necessary to metabolize the amino acid phenylalanine. Therefore, sustained high consumption of apm can promote seizures and subsequent brain damage in susceptible humans (Maher and Wurtman, 1987). However, this theory was contradicted by Stegink *et al.* (1980,

1981, 1987) indicating that a 100 mg/kg body wt. abuse dose of apm taken in a single dose was unlikely to have serious effects in those heterozygous for PKU. Methanol, in the methyl ester form, constitutes approximately ten percent of apm by weight. For example a 355 ml (one can) of a diet beverage containing aspartame will result in approximately 21 mg of methanol upon metabolization. Methanol occurs abundantly in the human diet. Baked potatoes, fruit juices and alcoholic beverages all contain methanol, often in larger concentrations than in apm. In the body, methanol is metabolized to formaldehyde which is rapidly degraded to formate (the factor responsible for methanol poisoning). Blood formate levels and urine excretion of formate were studied in adults administered a 200 mg/kg body wt. dose of apm (six times the estimated daily intake). This is equivalent to a consumption of more than 50 cans of apm-sweetened beverage at one time (Stegink *et al.*, 1983). The subjects showed no elevated levels of blood formate and exhibited rapid excretion of formate from the body. Infants given 100 mg/kg apm per day showed methanol levels similar to those in adults, indicating similar rates of metabolism and excretion.

Acute and chronic toxicology and carcinogenicity tests with rats, mice, hamsters, dogs and rhesus monkeys showed no significant effects or indications of toxicity or carcinogenicity

attributable to apm administration. Doses used were 13 g/kg body wt. in acute studies and 8 g/kg body wt. in chronic studies (Connolly *et al.*, 1978).

Pharmacology studies were performed on physiological systems such as the digestive and nervous systems to determine if apm had any biological activity other than that of creating the perception of sweet taste (Molinary, 1984). All investigations showed a total lack of pharmacological effect associated with apm, with the exception of an overload effect of phenylalanine when apm was given to weanling rats in very large amounts (Kessler and Clark, 1978).

Several metabolic studies observed and described the absorption, distribution, metabolism and excretion of apm by radiolabeled techniques. Aspartame is digested into its three moieties; phenylalanine, aspartic acid and methanol. These substrates are absorbed, metabolized and excreted by the same metabolic pathways, as when they occur naturally in other foods (London, 1988; Stegink *et al.*, 1983).

Through host-mediated, dominant, lethal and in vivo cytogenic mutagenicity assays, the potential of apm to produce mutation was studied. APM was not shown to alter the mutagenic frequency, and therefore does not appear to cause mutations (Kessler and Clark, 1978).

Similarly, all teratology studies showed that dietary administration of apm did not significantly effect fertility, the development of fetal abnormalities, mean gestational period, maternal survival, litter size or sex distribution of laboratory rats (Molinary, 1984).

Many toxicological evaluations of the major decomposition product of apm, diketopiperazine (DKP), have been performed. Because apm may contain very small amounts of DKP, and because apm may convert to DKP under certain storage conditions, the human population may consume small but varying amounts of this substance. The thorough evaluation of DKP through teratology, toxicology, mutagenicity and pharmacology tests has shown no adverse effects directly attributable to DKP up to doses of 3 g/kg body wt. (Ishii, *et al.*, 1981; Janssen and Heijden, 1988).

Studies have shown that apm is well tolerated by normal adults, adolescents and children in both short-term (6 weeks) and long-term (12 weeks) clinical evaluations. Further studies were carried out on diabetic and obese persons as these groups may potentially consume greater than average quantities of aspartame-containing foods (Horwitz, 1984). Similarly, lactating women were evaluated using a 50 mg/kg body wt. dose to determine if apm would effect the composition of human milk.

No significant effects were found in either test (Stegink *et al.*, 1979; Filer *et al.*, 1983).

In light of the contemporary interest of consumers in foods and food additives, it is not surprising that the amount of clinical trials and human studies on aspartame is unprecedented compared to any other food additive. Therefore, it can be safely concluded that the use of apm in various processed food should not pose any health hazards not encountered with other naturally occurring food components.

2.4. ACESULFAME-K

Many artificial sweeteners considered for use in foodstuffs have been discovered by chance and acesulfame-K is no exception. In 1967 Clauss and Jensen, researchers with Hoechst Co., a producer of chemicals and pharmaceuticals in Frankfurt, West-Germany, noticed a sweet taste while investigating compounds made by reacting butyne with fluorsulfonyl isocyanate (Anon., 1983). The sweet taste was due to 5,6-dimethyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide, a compound with a ring system which had not been synthesized previously.

Systematic research on dihydrooxathiazinone dioxides revealed a number of sweet tasting compounds in this group of

substances. Variations of substituents in positions 5 and 6 of the ring system showed noticeable influence on the purity and intensity of the sweetness. All the synthesized dihydro-oxathiazinone dioxides proved to be sweet, as well as the unsubstituted ring itself. The maximum sweetness was found with components that had short-chain alkyl groups. In addition to examining substituent influence on sweet flavor, variations were made within the ring structure. Studies have shown that altering the molecular structure of 5,6-dimethyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide, did not reveal any new sweet-tasting compounds. Even methylation on the nitrogen atom resulted in a non-sweet compound. Comparison of the different compounds clearly demonstrated that 6-methyl-1,2,3-oxathiazine-4(3H)- one-2,2-dioxide (acesulfame-K) exhibited the most favorable taste properties upon evaluation, including sensory trials, and proved to be the most suitable of the compounds for its purpose as sweetener (Rymon Lipinski and Lueck, 1981). The final structural formula of acesulfame-K is shown in Figure 2.5.

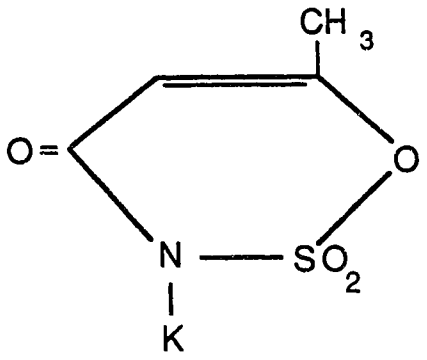

Figure 2.5. The structural formula of acesulfame-K (adapted from Clauss *et al.*, 1976).

During the first years of development of this sweetener the name acetosulfam was used, however, the name 'acesulfame potassium salt' was registered as the generic name by the World Health Organization in 1978. For simplicity reasons this name was abbreviated to 'acesulfame-K' shortly after. Table 2.5 lists alternative names used for the acesulfame potassium salt.

Although a few more intensely sweet dihydrooxathiazinone dioxides were synthesized, none were as pleasant-tasting as acesulfame-K. Also, this compound was comparatively easy to synthesize from acetoacetic acid derivatives and therefore it was selected for systematic stability, toxicity and acceptability studies.

Table. 2.5. Alternative names and their origins (where applicable) used for acesulfame potassium salt

Name	Origin
Acesulfame-K	commonly accepted
Potassium salt of 6-methyl-1,2,3-oxathiazine-4(3H)-one-2,2-dioxide	chemical terminology
Potassium salt of 3,4-dihydro-6-methyl-1,2,3-oxathiaxine-4-one-2,2-dioxide	"
HOE 0-95K	Hoechst
VPO-95K	"
Sunette®	Celanese Co.
Sweet One®	"
Acetosulfam	German
Acesulfam-K	"
Acesulpham	"

Source: Rymon Lipinski and Huddard, 1983.

2.4.1. Synthesis

The synthesis of acesulfame-K begins with acetoacetic acid

tert-butyl ester and fluorosulphonyl isocyanate. The end product of the reaction of these compounds is a-(N-fluorosulphonyl carbamoyl)-acetoacetic acid tert-butyl ester (Clauss and Jensen, 1973).

This compound is not stable, releasing CO_2 and isobutene, in its conversion to N-fluorosulphonyl acetoacetic acid amide. Fluorosulphonyl acetoacetic acid amide cyclizes with potassium hydroxide to form dihydrooxathiazinone dioxide. Due to the strong acidity of free oxathiazinone dioxide compounds, the potassium salt is produced directly.

Continuous production of acesulfame-K, starting with the synthesis of fluorosulphonyl isocyanate both on pilot and industrial levels has proven to be successful (Rymon Lipinski and Lueck, 1981).

2.4.2. Properties and Stability of Acesulfame-K

Acesulfame-K is a white, crystalline substance with particle size varying from powder to small crystals. Paulus (1975) determined the specific gravity of acesulfame-K to be 1.83 g/cm^3. Acesulfame-K appears to have a virtually unlimited shelf-life at room temperature as samples stored at normal ambient temperature (either exposed to or protected from light)

showed no sign of decomposition after several years. After a period of six to eight years, samples showed no difference in analytical composition, when compared to freshly produced material. Acesulfame-K, in its pure state, is non-hygroscopic, therefore under normal storage conditions can be kept for a long time. The melting point of acesulfame-K is not clearly defined, however, when heated slowly, decomposition can be observed at 225°C. On the other hand, the decomposition of acesulfame-K will occur at a much higher temperature when it is heated very rapidly. Thus, spray drying under normal conditions should not cause any decomposition of acesulfame-K in products containing this substance (Clauss *et al.*, 1976).

2.4.3. Sweetness

Acesulfame-K is about 200 times sweeter than sucrose and has a very similar sweetness intensity to aspartame (see Table 2.2). Acesulfame-K, like all high intensity sweeteners, has the greatest sweetness intensity at the threshold level with a decrease in intensity when concentration is increased. In acidic beverages and foods, the sweetness intensity of acesulfame-K appears to be increased whereas the sweetness of sucrose tends to be slightly masked (Lueck, 1981). At elevated temperatures,

acesulfame-K retains its sweetness, whereas a reduction in sweetness was found for sodium saccharin and sodium cyclamate in similar conditions (Hoppe and Gassmann, 1980).

2.4.4. Solubility

Acesulfame-K is readily soluble in water. At 20°C its solubility is approximately 270 g/L water, and with increased temperature (100°C) the solubility increases sharply, far above 1000 g/L. Therefore, at room temperature, the solubility of acesulfame-K is sufficient to produce concentrated stock solutions. Acesulfame-K is much less soluble in alcohol as only 1 g/L will dissolve in it at 20°C, while 100 g/L of acesulfame-K will dissolve in a mixture containing 50% ethanol and 50% water v/v. The pH of diluted solutions of acesulfame-K and water is neutral. No indication of undesired reactions such as turbidity or precipitation has been observed in food products containing acesulfame-K. The product can be purified easily by recrystallization enabling high purity acesulfame-K to be produced, to meet the requirements for food additives (Clauss *et al.*, 1976).

2.4.5. Applications and regulatory status

Acesulfame-K is suitable for use in a wide range of products, including low calorie and diabetic foods. It can also be used as a sugar substitute in chewing gum, desserts, and beverages (Rymon Lipinski and Lueck, 1983). Outside the food sector, acesulfame-K can be used to mask unpleasant flavor patterns and sweeten oral hygiene products and pharmaceuticals (Rymon Lipinski and Lueck, 1981). Upon completion of the toxicological programs, petitions for the approval of acesulfame-K as a food additive were filed in Canada, the US and many European countries. This resulted in the allocation of an acceptable daily intake (ADI) of 9.0 mg/kg body wt. by organizations such as the World Health Organization and the Food and Agriculture Organization (WHO, 1980). The use of acesulfame-K in certain food products has been approved in Europe and the United States; however in Canada the use is yet to be approved.

2.4.6. Safety

Extensive safety studies were carried out on acesulfame-K and concluded that the substance could be regarded as non-toxic. The oral LD_{50} (the dosage that resulted in the death of 50% of

the experimental animals after administration) of acesulfame-K was determined on rats to be 6.9 to 8 g/kg body weight.

A 90-day study was carried out on albino rats to detect potential subchronic toxic effects of acesulfame-K. The animals received concentrations of 0 - 10% w/w of acesulfame-K in their diets. To detect the potential carcinogenic effect of acesulfame-K, long-term feeding experiments were carried out on rats fed 3% acesulfame-K in their diets. As well, carcinogenic properties were studied in long-term experiments on mice and dogs. The results of these studies were negative and potentially opened the door for acesulfame-K as a high potency sweetener in specified food products (Rymon Lipinski, 1985; Anon., 1988).

Mutagenicity testing of acesulfame-K was carried out using various experimental approaches, all studies proving acesulfame-K to be non-mutagenic. The tests included a dominant lethal test, a micronucleus test, tests for malignant transformations, bone marrow investigations in hamsters, and DNA-binding (WHO, 1980).

In reproduction studies with rats fed acesulfame-K, it was shown that the reproductive behaviour of these animals was not affected. Also, no effect was found on the fertility and litter size of the animals or their offsprings birth weight, growth, and

mortality rate (WHO, 1980). No teratogenic effects were observed upon examination of the internal organs and skeletons of the fetuses from rat dams fed acesulfame-K during pregnancy.

The metabolism aspect of acesulfame-K appears to be entirely different from that of apm as there is no apparent breakdown of acesulfame-K in the digestive system of humans (WHO, 1980). Numerous investigations were carried out on rats, pigs, dogs, and human volunteers each concluding that after consumption, acesulfame-K is quickly eliminated from the digestive system, mainly via the urine, in an unaltered state (Rymon Lipinski, 1988). Similarly, no metabolic breakdown of acesulfame-K was detected in studies with radio-labelled acesulfame-K, leading investigators to conclude that acesulfame-K is toxicologically safe for human consumption (WHO, 1980).

2.5. THE FUTURE OF ASPARTAME AND ACESULFAME-K ON THE WORLD FOOD MARKET

Acesulfame-K was cleared by the FDA in July 1988 for use in various dry bases and as a sugar substitute. In the United States, acesulfame-K is being sold in retail markets under the Sweet One® label as a tabletop sweetener. The industrial

ingredient is being marketed under the Sunette® label. Current approval allows for its use in tableted sugar substitutes; instant coffee, tea and beverage bases; dry bases for gelatin, pudding and pudding desserts; dairy product analogs; and chewing gum. The manufacturer of acesulfame-K is currently pursuing approval for soft drinks and baked goods (Anon., 1989b). Clearance for use in confectionery and other products is pending. No special labeling is required unless the product is used for special dietary needs. No special health warning is required.

The future of apm and acesulfame-K on the world food market now depends on marketing and consumer acceptance. As a versatile high potency sweetener, apm already has been widely accepted in the weight-conscious developed world. However in light of the Delaney clause (Senti, 1988) which condemns any carcinogenic food or additive and following the scare of cyclamate and saccharin, many consumers are suspicious of the metabolic end products of APM. Within its seemingly lack of toxicity, this may be the niche into which the latest high potency sweetener, acesulfame-K, will fit.

Cyclamate and saccharin are widely used in Europe as sweeteners in whey-based beverages, however, since these two high potency sweeteners do not have a clean bill of health in North America, apm and acesulfame-K instead may be suitable

for use in these products. Therefore with these high potency sweeteners the sugar-reduced whey beverage may gain increased popularity by persons who are unable to consume sugar for health, dietary, or personal reasons.

3. MATERIALS AND METHODS

3.1. MATERIALS

3.1.1. Whey

During the course of this experiment, one 25 liter batch of cottage cheese whey was supplied by Palm Dairies Ltd. Edmonton each week and stored at 4°C. The pH of each batch of whey was approximately 4.3 and the total solids, 6.5%.

3.1.2. Fruit concentrates

Two separate fruit juice concentrates (mango and orange) were required for the whey beverage formulation:

a) Mango concentrate was supplied by Eurocitrus, Oosterhout, the Netherlands. This product had a Brix refractometric reading of 60° and was supplied as a combination of mango pulp and juice.

b) Orange concentrate was supplied by Palm Dairies Ltd., Edmonton. The Brix value of this product, which was a pure juice concentrate, was also 60°.

3.1.3. Sweeteners

Invert sugar syrup, aspartame and acesulfame-K were the three types of sweeteners used in this investigation.

Made from sugar beets, the invert sugar syrup was supplied by Palm Dairies Ltd., Edmonton and had a Brix refractometric reading of 67°. The aspartame was supplied by Searle & Co., Skokie, Ill., and the acesulfame-K by Hoechst Celanese, Somerville, N.J.

3.1.4. Enzyme for the lactose hydrolysis

As mentioned by Jackson and Jelen (1989), the enzyme, fungal ß-D-Galactoside galactohydrolase, was derived from the micro-organism Aspergillus oryzae. This acid lactase, in powder form, was purchased from E.D.C Enzyme Development, New York, NY.

3.1.5. Acidification of the whey

For all the studies a prototype model of a whey-based beverage made from fresh cottage cheese whey (pH 4.3), was

used. Anhydrous citric acid (Fisher Scientific Co. Fair Lawn, NJ.) was used to lower the pH to 3.6 - 3.7 thereby increasing the heat stability of the whey proteins (Patocka *et al.*, 1987; Jelen and Buchheim, 1984) and the palatability of the final product. The pH adjustment was controlled with an expanded scale pH-meter (model 320, Fisher Scientific Co. Fair Lawn, NJ.) and Orion pH-electrode (cat. no. 91-04, Orion Research, Boston, MA.).

3.2. Production of the beverages

A previously developed prototype formulation (Patocka, Dept. of Food Science, University of Alberta, personal communication) was modified for this research as shown in Tables 3.1.1 and 3.1.2.

All ingredients were weighed, combined with the acidified whey, and stirred until dissolved. Samples for the heat treatment experiment were divided into four 200 mL flasks (one unheated control), covered and put into a waterbath at 90°±1°C. The samples took eight minutes to reach 90°C after which they were subject to heat treatments of 2, 15, and 30 minutes respectively. Directly after the heat treatment, each sample was cooled in cold water and placed in a 4°C cooler. All samples were held for approximately twenty four hours prior to scheduled

sensory evaluations.

Whey used for the products containing hydrolyzed lactose was subjected to the procedure, described by Jackson and Jelen (1989), resulting in 90% hydrolysis in a batch process with acid lactase. The amount of hydrolysis in the whey was measured by HPLC analysis (Demaimay and Baron, 1981), at the Alberta Agriculture Food Laboratory (O. S. Longman Building, Edmonton).

Table 3.1. Original formulation of the whey-based fruit beverage prototype

Ingredient	Percentage (%) w/w
Juice concentrate	
Mango	5.0
Kiwi	3.0
Orange	1.5
Invert sugar (67°Brix)	10.5
Citric acid	0.3
Whey	66.7
Water	13.0
	100

Table 3.2 Adjusted formulation of the whey-based fruit beverage

Ingredient	Control (%) w/w	Experimental (%) w/w
Juice concentrate		
Mango	5.0	5.0
Orange	1.5	1.5
Citric acid	0.3	0.3
Invert sugar (67° Brix)	10.5	-
High potency sweetener	-	0.25*/0.275**
Whey	82.7	93
	100	100

* apm
** acesulfame-K

3.3. SENSORY EVALUATION

3.3.1. Screening of the panelists

In order to obtain a small, efficient taste panel, prospective panelists were screened based on their ability to detect the desired sensory differences in the food to be tested. In this project, where the detection of differences in sweetness was one of the main objectives, samples of a whey-based fruit beverage with differing levels of sweetness were presented to a large group of prospective panelists familiar with taste panel procedures. Based on their ability to discriminate among samples, panelists were selected following the completion of four triangle tests using an unheated whey-based beverage made according to the adjusted formula (Table 3.2). The difference between the samples in the triangle test was 0.005% apm on a weight basis, which is comparable to a 1.0% w/v difference in sugar addition. This is twice the threshold (minimal stimulus concentration) for sucrose (Larmond, 1977). Twenty-eight volunteers were given two triangle-tests (taste panel form Appendix 1) where there was no difference between sets A and B except the addition of a tasteless, odorless food colorant to all samples in set B. The test was repeated after twenty-four hours with the same panelists and products. Out of the twenty-eight original panelists, nine were consistently able to distinguish

between the test samples. These individuals were chosen as the pool of "qualified" panelists for the experimental investigations in this project. The actual number of panelists for the individual tests varied based on the needs and availability.

3.3.2. Taste panel procedures

After screening, panelists were trained to identify and quantify the sensory characteristics of the experimental products. Training sessions were focused on accurate detection of differences in sweetness levels in tests where sweetness was the only attribute. These tests included the determination of equisweetness, the synergistic effects of aspartame and acesulfame-K, and the effect of lactose hydrolysis in whey (Appendix 2, 'sweetness'). In the heat treatment study the attributes of sweetness, off-flavor and aftertaste were identified and quantified by the panelists. Because all the panelists were acquainted with the use of tasting procedures, a nine point descriptive rating scale (Larmond, 1977) was used for all the tests involving sweetness only as well as the combined sweetness-off-flavor-aftertaste determinations (Appendix 2). In each test, a reference representing the mid-anchorpoint was used and the panelists were asked to compare each sample to

this reference and to comment on each sample. Controllable characteristics such as sample temperature, size and appearance were standarized ensuring the consistency of each trial. The test area consisted of individual booths with colored (red) lighting to eliminate minor color differences in the product.

3.3.3. Statistical analyses

The results of all taste panel tests were analyzed statistically in order to obtain specific information regarding the effects of the treatment factors. These factors included: lactose hydrolysis, the use of different sweeteners, and the effects of heat. A combination of two types of statistical analysis; 2-way Analysis of Variance (ANOVA) and Duncan's test (Steel and Torrie, 1980) were considered to be the most useful in the analyses of the taste panel results.

3.4. TASTE PANEL TESTS

3.4.1. Equisweetness

The objective of this experiment was to determine the amount of aspartame and acesulfame-K required to obtain

sweetness identical to that imparted by the 10.5% invert sugar (equisweetness). In order to ascertain the required aspartame and acesulfame-K amounts, taste panels were carried out comparing the 10.5% invert sugar prototype whey beverage standard with whey beverages containing various levels of the artificial sweeteners. Comparison of the formulation containing four different levels of one artificial sweetener with the standard was made on a hedonic (nine-point), descriptive scale (Larmond, 1977) with 5.0 assigned as the equisweetness point (Appendix 1). Samples with scores of less than 5 were less sweet, while those with scores greater than 5 were sweeter than the standard. The number of panelists (n) was four and the number of replicates per experiment (reps.), three, for a total of 12 data replicates.

3.4.2. Synergistic effects between the high potency sweeteners

In this experiment, the objective was to confirm the presence of synergistic effects between aspartame and acesulfame-K in a whey-based fruit drink. Using the results of the equisweetness study, panelists compared five artificially sweetened samples with the standard reference sample

containing 10.5% invert sugar (67°Brix). The artificially sweetened samples were produced with: 100% aspartame (0.25 g/L); !00% acesulfame-K (0.275 g/L); 75:25 or 25:75 (apm: acesulfame-K), which means 75 percent of the equisweetness level of one sweetener combined with 25 percent of the equisweetness level of the other; and a 50:50 combination. The sweetness of the standard reference sample was again, rated 5.0.

3.4.3. Lactose hydrolysis in whey

The objective of this study was to determine the sweetness and synergistic effects of lactose hydrolyzed whey in the formulations with the various sweetening systems. Five variations of the original formula (0%, 25%, 50%, 75%, and 100% sweetener reduction) were tested against the reference formula for sweetness. The initial amount of each sweetener was based upon the results of the equisweetness-comparison test. Panelists were given five lactose-hydrolyzed samples and a non-hydrolyzed reference sample. For both the test samples and the reference the same sweetener, either invert sugar, apm, or acesulfame-K, was used, however, the amount of sweetener was reduced by 0%, 25%, 50%, 75%, and 100% in the lactose-hydrolyzed samples.

3.4.4. Heat stability study

The objective of this experiment was to determine the impact of heat on the stability of the artificial sweeteners in the acidic environment of this beverage and on the overall taste quality of the whey based fruit beverage. Identical 200 ml samples were placed in a 90°±1°C waterbath; the samples reached the waterbath temperature within eight minutes; after which time they were removed individually after further 2, 15, or 30 minutes respectively. Immediately after removal, the samples were cooled in cold water and placed in a 4°C cooler. Also, an unheated, invert-sugar-sweetened reference was included. Panelists compared the four samples to the reference sample for sweetness, off-flavor and aftertaste in a multiple comparison test (Larmond, 1977). The objective of this multiple comparison test was to determine whether there was any effect of heating of the beverage prototype on the attributes which were agreed upon during the training of the panelists; sweetness, off-flavor (specified as 'cooked milk') and aftertaste (specified as 'lingering sweetness'). The number of panelists (n) was nine and the number of replicates per experiment (reps.), three. The same 9-point scale was used as in the previous tests with 5.0 as

the 'equal-to-reference' anchor point.

3.5. HPLC-DETERMINATION OF ASPARTAME AND ACESULFAME-K

The objective of this analysis was to confirm analytically any possible losses of apm and acesulfame-K due to heat in the acidic environment of the whey beverage. For both sweeteners, apm and acesulfame-K, the average amount of sweetener remaining after each heat treatment at 90°C (2, 15, and 30 min., respectively) was determined. The method used, chosen for its accuracy and reproducibility, was High Performance Liquid Chromatography (HPLC). The equipment used for the analyses included: a Waters Programmable Multiwavelength HPLC Detector (Millipore Ltd., Mississauga, Ont., Canada); a SSI HPLC pump (model 222, Scientific Systems Incorporated, State College, PA., USA.); a Shimadzu Chromatopac printer (model C-R3A, Shimadzu Scientific Instruments, Inc. Colombia, MD., USA.).

3.5.1. Determination of aspartame by HPLC

For the determination of apm a C-18 Spherisorb (250 x 4.6 mm i.d., Phenomenex, Torraine, CA., USA) column was used with

the mobile phase consisting of 0.05M N-(n-Octyl)-dimethylamine (DMOA) in 2.0M H_3PO_4. The wavelength was set at 210 nm. (UV); the pressure was set at 2500 psi; the injection volume at 20 µL; the flow rate at 2.0 mL/min; with the analysis proceeding at room temperature (Fox, 1987).

3.5.2. Determination of Acesulfame-K by HPLC

A Spherisorb Amino (NH_2) (300 x 4.6 mm i.d., Phenomenex, Torraine, CA., USA) column was used for the determination of acesulfame-K. The mobile phase, which was found to be the most suitable for this analysis, consisted of a prepackaged 2% v/v phosphate buffer (Fisher Gram-Pac®, pH 6.86) dissolved in a 50% v/v ethanol-water mixture. The wavelength was set at 227 nm. (UV); the pressure was set at 2500 psi; the injection volume at 20 µL; the flow rate at 0.8 mL/min.; with the analysis proceeding at room temperature. This method was developed by Grosspietsch and Hachenberg (1980) and modified for the analyses of this specific product.

3.6. ENERGY VALUES

The objective of this analysis was to determine the caloric

values of the whey-based fruit drink formulations. Samples (5g) of all three basic formulations (sweetened with invert sugar, apm or acesulfame-K) were analyzed in triplicate with a bomb calorimeter (Ac-3000, Leco-corporation, St. Joseph, Michigan, USA); the analyses were based on the rapid burning of the product under pressure (420 psi) in pure oxygen. The heat liberated from the sample was measured and calculated in calories per 100 grams of the initial product.

4. RESULTS AND DISCUSSION

This chapter is divided into three parts: (1) the results of the taste panel experiments and their statistical analyses; (2) the results of the objective analytical tests; and (3) estimates of economic comparisons.

4.1. SUBJECTIVE ANALYSES

4.1.1. Determination of sweetener levels (Equi-sweetness)

Beverages sweetened with one of the artificial sweeteners (either apm or acesulfame-K), were compared to the standard sweetened with invert sugar to determine the equisweetness level for each.

After preliminary taste panels it was found that, compared with the 10.5% invert sugar, the equisweetness levels of both apm and acesulfame-K ranged between 0.20 and 0.35 g/L of the beverage. In order to find the exact equisweetness levels, samples with the amount of sweetener within this range were compared (Table 4.1) with the standard reference sample which had, as the mid-point score, a value of five (5.0).

Table 4.1. Determination of the equisweetness points of apm and acesulfame-K in a whey-based fruit beverage*

level of sweetener	average panel scores	
g/L	apm x ± sd	acesulfame-K x ± sd
0.20	4.25 ± 0.32	4.20 ± 0.26
0.25	5.00 ± 0.41	4.70 ± 0.34
0.30	6.25 ± 0.25	5.32 ± 0.20
0.35	6.38 ± 0.32	6.20 ± 0.34

*n=4, 3 reps. The assigned sweetness value of the reference sample was 5.

The taste panel results of apm were clear to distinguish, however, because the sweetness of acesulfame-K was often first perceived as 'bitterness', further training of the panelists was required. In addition, panelists have indicated that the sweetness of acesulfame-K takes longer to perceive, after tasting, than the other sweeteners, invert sugar and aspartame.

This delay in sweetness perception with acesulfame-K was also mentioned by Rymon Lipinski and Huddard (1983). The resultant equisweetness points for apm and acesulfame-K; 0.25 and 0.2[illegible] g/L, respectively, were used to further test the occurrence of synergism, the effect of lactose hydrolysis, and the effect of heat on beverages made with the two artificial sweeteners. These values are within the appropriate range as found in literature. As a guideline it is said that both apm and acesulfame-K are approximately 200 times sweeter than sugar, compared at sugar concentrations used for many food applications (Palmer, 1983). According to Yudkin (1986) apm has a slightly higher relative sweetness than acesulfame-K based on the comparison of threshold sweetness in a neutral aqueous solution.

4.1.2. The effect of lactose hydrolysis

The effects of lactose hydrolysis observed in the three beverage formulations sweetened with invert sugar, apm, or acesulfame-K, are shown in Table 4.2. The objective of this study was to determine whether the use of lactose hydrolysis could lead to a substantial decrease of the sweetener used. Each standard used for this study contained the same sweetener as

Table 4.2. The effects of lactose hydrolysis on sweetness in whey- based beverages, using three different sweeteners*

% reduction of sweetener	mean scores for sweetness in formulations containing		
	invert sugar	apm	ac-K
	x ± sd	x ± sd	x ± sd
0	4.88 ± 0.13[a]	5.38 ± 0.38[a]	7.00 ± 0.20[b]
25	4.25 ± 0.32[a]	5.38 ± 0.24[a]	6.25 ± 0.32[b]
50	3.25 ± 0.25[b]	4.00 ± 0.29[b]	4.88 ± 0.43[a]
75	2.50 ± 0.20[c]	3.00 ± 0.65[bc]	3.75 ± 0.32[c]
100	2.00 ± 0.00[c]	2.00 ± 0.20[c]	2.38 ± 0.13[d]

*n=4, 3 reps. Data in each column sharing identical letters are not significantly different ($p \geq 0.05$) from each other. Superscript[a] identifies no significant difference ($p \geq 0.05$) from the control which was assigned the value of 5.

experimental sample; however, the controls were made from whey with no prehydrolization of lactose to 90%, the sweetness

being set at 5. A score higher than 5 means the sample was perceived sweeter than the standard, a lower score means that the panelists perceived the sample as less sweet than the standard. The levels of the sweeteners in the artificially sweetened samples included: 100% aspartame (0.25 g/L); 100% acesulfame-K (0.275 g/L); 75:25 (apm:acesulfame-K), which means 75 percent of the equisweetness level of apm combined with 25 percent of the equisweetness level of acesulfame-K; and similarly, 25:75, and 50:50.

When the amount of invert sugar, added to a whey beverage made with hydrolyzed lactose whey, was reduced by twenty-five percent (w/w), no decrease of sweetness was detectable at the ninety-five percent confidence level. Also, when the amount of added apm was reduced by twenty-five percent (w/w), the decrease in sweetness was not detected by the taste panelists at the ninety-five percent confidence level.

When the amount of added acesulfame-K was reduced by twenty-five percent (w/w) and even fifty percent (w/w), no decrease in the level of sweetnesss was detected at the ninety-five percent confidence level. Additionally, the samples with zero percent (w/w) and twenty-five percent (w/w) reductions of acesulfame-K were rated significantly sweeter than the unhydrolyzed reference at the ninety-five percent

confidence level. Conversely, the samples with a seventy-five percent and one hundred percent reduction of acesulfame-K were both rated less sweet than the reference at the ninety-five percent confidence level.

It appeared that when either of the high potency sweeteners, apm or acesulfame-K, was used in combination with lactose-hydrolyzed whey, the amount of added sweetener could be reduced by at least twenty-five percent maintaining the level of sweetness in accordance with the reference sample.

4.1.3. Synergistic effects

Synergism between sweeteners results in an increase of sweetening power when certain sweeteners are combined (Porter, 1983). More specifically, the sweetness of each artificial sweetener appears to be enhanced by the presence of the other. The synergistic effects between apm and acesulfame-K were clearly established in this investigation with whey-based fruit beverages as shown in Table 4.3.

At the ninety-five percent confidence level ($p=0.05$), products containing a mixture of the two sweeteners were rated sweeter than the individual sweeteners themselves in each of the combinations used. This implies that synergistic effects

Table 4.3. The synergistic sweetness effect of combined and acesulfame-K in the whey-based fruit beverage*

sweeteners and their combinations	panel score x ± sd
100% apm**	4.88 ± 0.24[a]
100% acesulfame-K**	4.95 ± 0.57[a]
75Ac-K:25apm	6.50 ± 0.46[b]
50Ac-K:50apm	6.50 ± 0.35[b]
75apm:25ac-K	6.38 ± 0.43[b]

*n=4, 3 reps. Data in each column sharing identical letters are not significantly different ($p \geq 0.05$). The reference sample score was 5.0; superscript[a] indicates no significant difference ($p \geq 0.05$) from the control.

**100% apm= 0.25 g/L; 100% acesulfame-K = 0.275 g/L.

exist between apm and acesulfame-K making it possible to reduce the total amount of each sweetener in a product by twenty-five to fifty percent, when a combination of apm and acesulfame-K is used. These results imply that one could significantly reduce the costs of the total amount of sweetener used, if the costs of the two products would be comparable.

4.1.4. The effect of heat treatment

APM and acesulfame-K are heat stable at elevated temperatures in model systems such as mentioned in literature (Homler *et al.*, 1987; Rymon Lipinski, 1988), however, it was necessary to study the heat stability of these sweeteners in a whey-based beverage, as heat stability may be influenced also by the type of medium (Blenford, 1987). In addition, it was important to study the overall effect of the heat treatment on the quality of the whey beverage. The results of these tests are compared within the following three attributes: sweetness; off-flavor; aftertaste. Tables 4.4 - 4.7 list the means of the panel scores for each of the sweeteners invert sugar, apm, and acesulfame-K, compared for each of the attributes sweetness, off-flavor, and aftertaste. Also, a non-sweetened sample was included to determine whether the flavor effects were caused by factors other than the sweeteners themselves. The heat treatment used in this study (90°C) was intended to be more severe than any High Temperature Short Time (HTST) or pasteurization procedure, since it is expected that the preferred industrial treatment will be similar as that used typically for the extended shelf-life of fruit juices. An unheated sample

Table 4.4. The sensory effects of heat treatment in an invert sugar-sweetened, whey-based fruit beverage*

Heat treatment min. at 90°C.	Sensory Characteristics		
	sweetness x ± sd.	off-flavor x ± sd.	aftertaste x ± sd.
0	4.97 ± 0.25[a]	5.07 ± 0.12[a]	5.23 ± 0.19[a]
2	5.33 ± 0.19[a]	6.08 ± 0.20[b]	5.64 ± 0.20[a]
15	5.22 ± 0.21[a]	6.46 ± 0.20[b]	5.61 ± 0.23[a]
30	5.03 ± 0.17[a]	6.30 ± 0.31[b]	5.26 ± 0.10[a]

*n=9, 3 reps. The reference used was a non-heated sample, identical to the "0 min. heat-treatment". Data in each column sharing identical letters are not significantly different ($p \geq 0.05$). Superscript[a] signifies no significant difference ($p \geq 0.05$) from the control.

(identical to the reference sample) was included in each experimental set.

Upon comparison of the panel scores, it appeared that none of the heat treatments significantly affected the perceived amount of sweetness or aftertaste. The results of the attribute 'off-flavor' did change, however. In the four formulations

Table 4.5. The sensory effects of heat treatment on an APM-sweetened, whey-based fruit beverage*

Heat treatment min. at 90°C.	Sensory Characteristics		
	sweetness $\bar{x} \pm sd$	off-flavor $\bar{x} \pm sd$	aftertaste $\bar{x} \pm sd$
0	5.19 ± 0.16^a	5.26 ± 0.27^a	5.37 ± 0.15^a
2	5.12 ± 0.17^a	6.13 ± 0.16^b	5.52 ± 0.13^a
15	5.08 ± 0.13^a	6.32 ± 0.34^b	5.44 ± 0.23^a
30	5.19 ± 0.18^a	6.97 ± 0.36^b	5.70 ± 0.25^a

*see footnote, Table 4.4.

including the sweeteners invert sugar, apm, and acesulfame-K, as well as the non-sweetened sample, the heat treatments increased the off-flavor, this difference (shown in Tables 4.4 - 4.7) was significant at the ninety-five percent confidence level.

Table 4.6. The sensory effects of heat treatment on an acesulfame-K- sweetened, whey-based fruit beverage*

Heat treatment min. at 90°C.	Sensory Characteristics		
	sweetness $\bar{x} \pm sd$	off-flavor $\bar{x} \pm sd$	aftertaste $\bar{x} \pm sd$
0	4.66 ± 0.28^a	5.48 ± 0.21^a	5.22 ± 0.15^a
2	4.96 ± 0.28^a	6.28 ± 0.17^b	5.27 ± 0.15^a
15	4.89 ± 0.23^a	6.49 ± 0.26^b	5.07 ± 0.07^a
30	4.62 ± 0.11^a	6.84 ± 0.25^b	5.14 ± 0.10^a

*see footnote, Table 4.4.

Table 4.7. The sensory effects of heat treatment on a non-sweetened, whey-based fruit beverage*

Heat treatment min. at 90°C.	Sensory Characteristics		
	sweetness x ± sd	off-flavor x ± sd	aftertaste x ± sd
0	4.60 ± 0.19[a]	5.43 ± 0.15[a]	5.04 ± 0.10[a]
2	4.74 ± 0.16[a]	6.58 ± 0.38[b]	5.21 ± 0.11[a]
15	5.11 ± 0.22[a]	6.48 ± 0.23[b]	5.26 ± 0.22[a]
30	4.91 ± 0.19[a]	6.78 ± 0.38[b]	5.21 ± 0.11[a]

*see footnote, Table 4.4.

It can be assumed that since the 'off-flavor' occurred in each of the formulations, including the non-sweetened control, the

effect was caused by heating the whey, not by the individual sweeteners.

4.2. OBJECTIVE ANALYSES

4.2.1. The effect of heat treatment on apm and acesulfame-K

The objective of this study was to determine analytically any possible minor losses of apm and acesulfame-K due to the heat treatment applied to the whey-based beverage, which is a strongly acidic environment. (This was the same beverage as was used for the taste panels).

Table 4.8 shows that aspartame was only slightly affected by heating of this product. Even after thirty minutes at 90°C, 87 percent of the initial amount of apm remained in the product, while acesulfame-K was found unaffected by heat. These results are in agreement with literature; around pH 4.1 apm is known to be most heat resistant (Beck, 1978). In this system and with the application of these heat treatments, the amount of degradation was so small that it could not be detected by the panelists. As far as acesulfame-K is concerned, according to literature, no degradation of this sweetener should occur, even after very

prolonged heating (Crosby and Wingard, 1979).

Table 4.8. The heat stability of apm and acesulfame-K in a whey-based fruit beverage*

Heat treatment (min. at 90°C)	Amount remaining			
	apm		acesulfame-K	
	g/L	%	g/L	%
0	0.250	100	0.275	100
2	0.228	91.1	0.272	100
15	0.222	88.8	0.273	100
30	0.217	86.8	0.274	100

*3 reps. The amount of sweetener present in the non-heated sample (0 min.) is equivalent to that in the reference sample used in the taste panels.

4.2.2. Energy values

The energy values of the various formulations of the whey-based fruit beverages as measured using a calorimeter are shown in Table 4.9. Since apm was present in such a low concentration, it did not subtantially add to the number of calories.

Table 4.9. Measured caloric values of the whey-based beverage formulations

Formulation (Sweetener)	kcal/100mL
Invert sugar (67° Brix)	61.9
APM-sweetened	36.7
Acesulfame-K	36.2

The presence of sugar in the sugar-sweetened formulation approximately doubled the calories present in the beverage. The difference in caloric value between the apm-sweetened and the acesulfame-K-sweetened products were small as, theoretically, apm only added 0.025 g/100 mL x 4 kcal/g = 0.1 calories, as

compared to acesulfame-K with no calories. The reason why the amount of calories provided by whey is slightly higher in the experimental products (see Table 4.10), sweetened with the high potency sweeteners, is due to the fact that these do not add bulk to the products, like sugars do, therefore these products contain slightly more whey. Since with the use of high potency sweeteners the amount of calories in the product can be reduced by fifty percent, it is now possible, using modern technology, to manufacture a product in line with todays' consumer demands.

Table 4.10. Calculated contributions of ingredients to total caloric values of the whey-based beverages

	Energy (kcal/100 mL)	
Ingredient	Control	Experimental
whey	19.0	21.4
invert sugar	28.1	-
fruit concentrates	15.6	15.6
total	62.7	37.0

4.3. ECONOMIC COMPARISONS

The objective of this estimation was to compare the cost of lactose hydrolysis in whey with the amount of sweetener required to provide the desired sweetness.

As shown in chapter 4.1.2, the amount of apm and acesulfame-K necessary to sweeten a whey-based beverage could be reduced by up to 50 percent, if lactose-hydrolyzed whey was used. Therefore, it was important to compare the process of lactose hydrolysis with the use of high potency sweeteners and to determine which was more economically feasible.

Initially, the costs of the traditional process (no lactose hydrolysis and no high potency sweetener) had to be calculated and with this information, the maximum allowable cost of the alternative processes, established.

With the current cost of invert sugar being 0.436 dollars per kg and 105 g/L required to sufficiently sweeten the whey-based fruit beverage, 4.52 cents/L were added to the cost of the product due to the sweetener. Depending on the enzyme cost/hydrolysis time compromise considered to be the most appropriate, the cost of the hydrolysis step in the manufacture of a whey-based fruit beverage would be in the order of 3 - 4 cents per litre of hydrolyzed whey (Bernal, 1984). Another valid

comparison would be invert sugar vs. lactose hydrolysis, but, as shown in Table 4.2, the hydrolysis of lactose increases the perceived sweetness in this beverage by only twenty-five percent. Therefore, there is no economic advantage to the use of lactose hydrolyzed whey, in combination with invert sugar. For this reason, if the cost of the high potency sweeteners are substantially more than that of invert sugar, the process of lactose hydrolysis should be considered. The estimated cost of apm (Blenford, 1987) is 260 dollars per kg, and since 0.25 grams of this sweetener are required per liter of the beverage, it will add 6.5 cents to the cost of one liter of product. The difference in cost between invert sugar and the high potency sweeteners, at present, may warrant the use of lactose hydrolysis, if this could result in overall reduction of the amount of high potency sweetener. So, if the process of lactose hydrolysis would cost 3 cents per liter whey, and if we could reduce the amount of apm added to the beverage by fifty percent, this would be cheaper than 6.5 cents per liter. However, if only twenty-five percent reduction of the amount of apm is possible and/or if the cost of lactose hydrolysis would be 4 cents per liter whey, this step can not be considered economically feasible. The cost of the sweetener acesulfame-K is about a fifth of the cost of apm (Blenford, 1987) therefore, regarding the equisweetness level of

0.275 g/l, this sweetener will add approximately 1.4 cents to the cost of one liter of the whey-based beverage. This is so low that the process of lactose hydrolysis would have to cost less than 0.5 cents per liter whey for an economical advantage. Therefore the use of acesulfame-K as the primary sweetener is, compared to the process of lactose hydrolysis as well as to the use of invert sugar or APM, economically the most suitable. As mentioned before, Acefulfame-K for the use in food products has been approved in Europe and the United States, however the approval for use use in Canada has not been granted yet by Health and Welfare Canada.

5. SUMMARY OF RESEARCH FINDINGS, CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

5.1. SUMMARY OF RESEARCH FINDINGS AND CONCLUSIONS

Investigation of the flavor aspects using apm and acesulfame-K as sugar replacements in cottage cheese whey-based fruit drinks indicated that these sweetening agents were both suitable. The difference in caloric values between the apm and the acesulfame-K sweetened products was not significant. Very small amounts of aspartame and acesulfame-K were required to produce an acceptable sweetness level, reducing the amount of calories in the final product by fifty percent. When aspartame and acesulfame-K were combined their synergism reduced the amount of sweetener required by twenty-five percent. Furthermore, the enzymatic hydrolysis in whey reduced the amount of sweetener by an additional twenty-five to fifty percent. As described in Chapter 4.3, the process of lactose hydrolysis may or may not be economically feasible, depending on the costs of other ingredients such as the intense sweeteners and the lactase, where prices very often depend on the quantities of these products ordered. During the

heat treatments, a significant increase in 'off-flavor', was detected; the cause of this off-flavor was presumed to be related to the heat-induced changes in the whey proteins, rather than the sweeteners. Only a very slight loss of aspartame was analytically detected following the various heat treatments while no heat loss of acesulfame-K was found in the acesulfame-K-sweetened samples. The comments given by the taste panelists indicated that they preferred the invert sugar- and apm-sweetened samples over those sweetened with acesulfame-K due to a perception of slight bitterness found in the samples sweetened with acesulfame-K. Evaluation of all these factors should enable the processor to find the most suitable option under given conditions.

5.2. FOCUS ON FURTHER RESEARCH

The sensory studies of the whey based fruit beverages sweetened with aspartame, acesulfame-K or a combination of these high potency sweeteners indicated that these products could be considered marketable items in North America, taking economical as well as nutritional considerations into account. In this country, where frozen juice concentrates are popular due to their convenience, an investigation on whey concentrates for use

in these types of beverages would be recommended. It is assumed that when freezing processes are employed, crystallization of the lactose present in concentrated whey may occur causing 'sandiness'. Therefore, lactose hydrolysis could play an important role, eliminating this defect in the thawed product. In addition, it is believed that the use of a combination of juice concentrates would mask defects occurring during the heat treatment step, such as cooked flavor. Further research towards eliminating this defect would be advantageous. Further possibilities including flavors such as tropical fruit, chocolate, or vanilla, should be evaluated for their consumer appeal. Additionally, a UHT-version as well as carbonation of the whey-based beverage should be examined as two potential avenues that could help the whey drinks to penetrate the soft drink market.

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Appendix 1. Triangle Test Form for screening of panelists

Name: ----------

Date: ----------

Triangle-test for sweetness of breakfast-drink.

Group A.

Two samples are the same: please circle the odd sample.

____ ____ ____

Which sample is sweeter: odd sample sweeter ---

duplicates sweeter----

Group B.

Two samples are the same: please circle the odd sample.

____ ____ ____

Which sample is sweeter: odd sample sweeter---

duplicates sweeter---

Comments:

Appendix 2. Nine point descriptive rating scale

NAME: DATE:

You are receiving samples of breakfast-drink to compare for SWEETNESS, OFF-FLAVOR and AFTERTASTE. You have been given a reference sample, marked 'R', with which you are to compare each sample. Taste each sample; determine whether it has more, equal or less SWEETNESS, OFF-FLAVOR and AFTERTASTE than the reference. Then mark the AMOUNT of difference that exists.

	SWEETNESS				
Sample number	____	____	____	____	____
Sweeter than 'R'	____	____	____	____	____
Equal to 'R'	____	____	____	____	____
Less sweet than 'R'	____	____	____	____	____
AMOUNT OF DIFFERENCE:					
None	____	____	____	____	____
Slight	____	____	____	____	____
Moderate	____	____	____	____	____
Much	____	____	____	____	____
Extreme	____	____	____	____	____

	OFF-FLAVOR ('cooked milk')				
Sample number	____	____	____	____	____
More off-fl. than 'R'	____	____	____	____	____
Equal to 'R'	____	____	____	____	____
Less off-fl. than 'R'	____	____	____	____	____
AMOUNT OF DIFFERENCE:					
None	____	____	____	____	____
Slight	____	____	____	____	____
Moderate	____	____	____	____	____
Much	____	____	____	____	____
Extreme	____	____	____	____	____

AFTERTASTE ('lingering sweetness')

Sample number	____	____	____	____	____
More aftert. than 'R'	____	____	____	____	____
Equal to 'R'	____	____	____	____	____
Less aftert. than 'R'	____	____	____	____	____
AMOUNT OF DIFFERENCE:					
None	____	____	____	____	____
Slight	____	____	____	____	____
Moderate	____	____	____	____	____
Much	____	____	____	____	____
Extreme	____	____	____	____	____

COMMENTS: